U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB-269 934

Acute Toxicity of Kepone (Trademark) to Four Estuarine Animals

Environmental Research Lab, Gulf Breeze, Fla

1977

TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing) 804.218, 3014.3

1 REPORT NO. EPA-600/3-77-030	2 JOURNAL ARTICLE	3 PB 269 934
4 TITLE AND SUBTITLE: ACUTE TOXICITY OF KEPONE TO FOUR ESTUARINE ANIMALS		5 REPORT DATE 6 PERFORMING ORGANIZATION CODE
7 AUTHOR(S): Steven C. Schimmel and Alfred J. Wilson Jr.		8 PERFORMING ORGANIZATION REPORT NO
9 PERFORMING ORGANIZATION NAME AND ADDRESS Same as Box 12		10 PROGRAM ELEMENT NO 11 CONTRACT/GRANT NO 1EA615
12 SPONSORING AGENCY NAME AND ADDRESS Environmental Research Laboratory, Gulf Breeze Office of Research and Development U. S. Environmental Protection Agency Gulf Breeze, Florida 32561		13 TYPE OF REPORT AND PERIOD COVERED 14 SPONSORING AGENCY CODE EPA/600/04

15 SUPPLEMENTARY NOTES CONTACT: S.C. Schimmel, 8-686-9011 (Gulf Breeze, FL)
REFERENCE: Chesapeake Science Vol. 18, No.2, 224-227, June 1977

16 ABSTRACT

Recent contamination of the James River estuary, Virginia, with Kepone prompted acute flow-through bioassays to determine the 96-hour toxicity of the insecticide to four estuarine species native to that ecosystem. The species and their 96-hour LC50 values were: grass shrimp (Palaemonetes pugio), 121 µg/liter; blue crab (Callinectes sapidus), >210 µg/liter; sheepshead minnow (Cyprinodon variegatus), 69.5 µg/liter; and spot (Leiostomus xanthurus) 6.6 µg/liter. Surviving animals were analyzed for Kepone. Average bioconcentration factors (the concentration of Kepone in tissues divided by the concentration of Kepone measured in seawater) were: grass shrimp, 698; blue crab, 8.1; sheepshead minnow, 1,548; and spot, 1,221.

17 KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

REPRODUCED BY NATIONAL TECHNICAL INFORMATION SERVICE U.S. DEPARTMENT OF COMMERCE SPRINGFIELD, VA. 22161

18 DISTRIBUTION STATEMENT: release to public	19 SECURITY CLASS (This Report): unclassified 20 SECURITY CLASS (This Page): unclassified	21 NO. OF PAGES: 5 22 PRICE: PC A02, MF A01

EPA Form 2220-1 (9-73)

Acute Toxicity of Kepone® to Four Estuarine Animals[1]

ABSTRACT: Recent contamination of the James River estuary, Virginia, with Kepone prompted acute flow-through bioassays to determine the 96-hour toxicities of the insecticide to four estuarine species native to that ecosystem. The species and their 96-hour LC50 values were: grass shrimp (*Palaemonetes pugio*), 131 µg/liter; blue crab (*Callinectes sapidus*), 2,110 µg/liter; sheepshead minnow (*Cyprinodon variegatus*), 69.3 µg/liter; and spot (*Leiostomus xanthurus*) 6.6 µg/liter. Surviving animals were analyzed for Kepone. Average bioconcentration factors (the concentration of Kepone in tissues divided by the concentration of Kepone measured in seawater) were: grass shrimp, 698; blue crab 8.4; sheepshead minnow, 1,548; and spot, 1,321.

Introduction

Few published data are available on Kepone toxicity to estuarine animals. Butler (1963) reported LC50 values (based on mortality or loss of equilibrium in 48 hours for shrimp and on inhibition of shell deposition in 96 hours for oysters) of 85 µg/liter for brown shrimp (*Penaeus aztecus*); 57 µg/liter and 15 µg/liter for eastern oysters (*Crassostrea virginica*) exposed at seawater temperatures of 14°C and 31°C, respectively. Twenty percent of the blue crabs (*Callinectes sapidus*) exposed to 1,000 µg/liter Kepone died in 48 hours. Butler's data were derived from flow-through bioassays and based on nominal, not measured, concentrations of Kepone in seawater.

Recent discharge of the insecticide, Kepone®, into the James River estuary, Virginia has resulted in contamination of that system's water, sediment, and biota. This contamination raised questions about the acute and chronic effects of Kepone on the aquatic life in the estuary and the potential danger to humans by eating contaminated animals.

In January 1976, we initiated flow-through bioassays to determine bioconcentration and acute toxic effects of Kepone on representative species found in the James River estuary. These were grass shrimp (*Palaemonetes pugio*), blue crab (*Callinectes sapidus*), sheepshead minnow (*Cyprinodon variegatus*), and spot (*Leiostomus xanthurus*).

Methods and Materials

Acute toxicity was determined by exposing 20 animals per aquarium to different concentrations of Kepone for 96 hours in flow-through bioassays similar to those described by Eaton et al (1978). All test animals were acclimated to laboratory conditions for at least ten days prior to testing. The temperature and salinity of seawater in which they were held were allowed to vary with those of Santa Rosa Sound, Florida, during acclimation and testing. Our acclimation and testing procedures were compatible with those of Standard Methods (APHA 1971). All test animals were captured in the vicinity of the Gulf Breeze Laboratory and samples contained no detectable Kepone (< 0.02 µg/g). During acclimation, test animals were fed frozen brine shrimp daily. Animals were not fed during the tests, but could obtain food (plankton and other particulate matter) from the unfiltered seawater. Seawater was pumped from Santa Rosa Sound into a constant head trough in the laboratory and delivered to each 18 liter aquarium by a calibrated siphon that delivered approximately 68 liter/hr. One control and five experimental aquaria were used in each test. Stock solutions of Kepone (88% pure), in reagent-grade acetone, were metered into experimental aquaria at the rate of 60 ml/hr.

The 96-hour LC50 values were determined for both nominal and measured concentrations of Kepone in seawater. Nominal concentrations were those calculated to be in seawater, based on the concentration of the stock solution, plus the stock solution and seawater flow rates. The LC50 values were based on measured Kepone concentrations determined by chemical analysis of the exposure water. Mortality data were subjected to probit analysis to determine LC50 values and their 95% confidence limits (Finney 1971).

At the end of each 96-hour test, surviving animals from each concentration were sacrificed, rinsed with acetone, and pooled as a single sample for residue analysis.

Water samples were analyzed by extracting one liter of seawater (twice with 100 ml of methylene chloride). The combined extracts were concentrated to about 5 ml in a Kuderna-Danish Concentrator on a steam table. Fifteen milliliters of benzene were added and the extract reconcentrated to remove the methylene chloride. The extract was cleaned up on a Florisil Column as described below.

Tissues of shrimp, crabs, or fish were weighed in 150 mm × 25 mm (OD) screw-top test tubes and extracted twice with 5 ml volumes of acetonitrile for 30 seconds with a model PT 10-35 Willems Polytron (Brinkman Instruments, Westbury, New York). The mixture was centrifuged and the acetonitrile transferred to a 250-ml separatory funnel. After the second extraction, the tissue was extracted with one 5-ml and one 10-ml volume of acetone. After each acetone extraction the tube was centrifuged and the acetone added to the 250-ml separatory funnel. To the combined extracts, 100 ml of 2.0% aqueous sodium sulfate and 10 ml of 1:1 diethyl ether-petroleum ether were added. The separatory funnel was shaken for one minute. After the solvent phases had separated, the lower aqueous phase

® Registered Trademark for decachlorooctahydro-1,3,4-metheno-2H-cyclobuta (cd) pentalen-2-one. Allied Chemical Company, 40 Rector Street, New York, New York 10006. Mention of commercial products does not constitute endorsement by the Environmental Protection Agency.

[1] Contribution No. 391, Environmental Research Laboratory, Gulf Breeze.

TABLE 1. Toxicity of Kepone to and uptake by four estuarine organisms after 96 hours exposure.

Species	Water Concentration (µg/liter) Nominal	Measured	Mortality (%)	Whole Body Residue (µg/g wet weight)	Bioconcentration Factor
Grass shrimp (*Palaemonetes pugio*)	Control	Nil*	0	Nil	–
	13.8	12	0	9.1	833
	24	18	0	18	933
	42	39	0	29	744
	75	69	5	42	609
	135	121	90	94	777
					$\bar{x}$ = 698
Blue crab (*Callinectes sapidus*)	Control	Nil	0	Nil	–
	42	–	0	–	–
	75	–	10	–	–
	135	110	5	0.85	7.7
	187	164	0	1.7	10.4
	240	210	0	1.3	6.2
					$\bar{x}$ = 8.1
Sheepshead minnow (*Cyprinodon variegatus*)	Control	Nil	0	Nil	–
	10	7.1	0	11.2	1577
	18	14	0	20.9	1493
	32	33	5	44.4	1030
	56	51.5	20	65.6	1235
	100	78.5	65	118.4	1508
					$\bar{x}$ = 1548
Spot (*Leiostomus xanthurus*)	Control	Nil	0	Nil	–
	7.4	1.5	5	1.7	1133
	13	3.4	10	3.2	941
	25	4.4	45	7.0	1591
	43.5	7.8	40	10.8	1385
	74	15.9	95	16.8	1057
					$\bar{x}$ = 1221

* Nil = non detectable; <0.02 µg/liter in water, <0.02 µg/g in tissue.

TABLE 2. 96-hour toxicity of Kepone to several estuarine animals in flowing seawater bioassays. The 95% confidence intervals are in parentheses. Animal sizes are rostrum-telson length for shrimps, carapace width for crabs, and standard length for fishes.

Species	Size (± mm)	Nominal 96-hour LC50 in µg/liter (95% Confidence Limit)	Measured 96-hour LC50 in µg/liter (95% Confidence Limit)	Temperature (± °C)	Salinity (± ‰)
Grass shrimp (*Palaemonetes pugio*)	37.8	154.8 (114.1-193.9)	120.9 (103.0-171.6)	20.0	16.0
Blue crab (*Callinectes sapidus*)	34.5	>240	>210	19.0	20.0
Sheepshead minnow (*Cyprinodon variegatus*)	20.0	83.0 (67.9-115)	69.8 (56.3-99.5)	18.0	15.0
Spot (*Leiostomus xanthurus*)	33.9	10.8 (8.3-14.0)	9.6 (5.3-8.8)	25.0	18.0

was drained into a 250-ml beaker and the upper ether layer was collected in a 25-ml Kuderna-Danish concentrator tube. The ether extraction was repeated three times with 5 ml of 1:1 diethyl ether petroleum ether. The combined extracts were concentrated just to dryness by placing the concentrator tube in a water bath at 45°C and blowing off the solvent with a gentle stream of nitrogen. The residue was transferred to a 200 mm × 9 mm (I.D.) Chromaflex column (Kontes Glass Co.) containing 2.5 gm of Florisil topped with 2.0 gm of anhydrous sodium sulfate. The column initially was washed with 10 ml of hexane and the residue trans-

ferred with four 0.5 ml volumes of 5% diethyl ether in hexane. The column was eluted with 20 ml of 5% diethyl ether in hexane to remove PCB and pesticides. Kepone was eluted in a second elution of 40 ml of 1% methanol in benzene. Extracts were concentrated or diluted to appropriate volumes for analyses by electron capture gas chromatography.

Determinations were obtained by Varian Aerograph Model 2100 and 1400 Gas Chromatographs equipped with 183 cm × 2 mm (I.D.) glass columns packed with 1% SP2100 and 0.75% SP2250, 0.075% SP2401 on 100/120 mesh Supelcoport. The operating parameters were: oven temperature 185°C, injector temperature 200°C, detector temperature 210°C, and nitrogen carrier gas flow rate 25 ml/min.

The average recovery rate of Kepone from fortified tissue was 87%; from water, 85%. Residue concentrations were calculated on a wet-weight basis without a correction factor for percentage recovery. All samples were fortified with an internal standard (dichlorobenzophenone) prior to analysis to evaluate the integrity of the results.

Results and Discussion

Kepone, at concentrations tested, was acutely toxic to shrimp and fishes but not to blue crabs. The LC50 values varied widely among species. Spot were the most sensitive with a 96-hour LC50 of 9.6 μg/liter; the sheepshead minnow LC50 was over ten times higher (169.5 μg/liter). The two crustaceans were less sensitive. Grass shrimp LC50 was 120.0 μg/liter, and no significant mortality was observed in blue crabs at measured concentrations as high as 210 μg/liter (Tables 1 and 2).

Although the sensitivity of fish to Kepone toxicity differed, the symptoms of Kepone poisoning were similar. An early symptom was lethargic behavior followed by loss of equilibrium. These symptoms occurred in sheepshead minnows at 48 hours in 56 and 100 μg/liter concentrations and 96 hours in 18 and 32 μg/liter concentrations. Spot exhibited the symptoms in 48 hours when exposed to 7.5, 13.5 and 24 μg/liter Kepone. An advanced stage of poisoning was evident in dark coloration of portions of the fish's body. This color change was striking in that some fish had normal coloration on one side of their bodies, while the other side was nearly black with a sharp line of demarcation. Some spot and sheepshead minnows were darkened in only one quadrant of the body; for example, the left side posterior to the pectoral fins. These color changes were always more marked and appeared earlier in the higher Kepone concentrations. Hansen et al. (1977) also noted color changes in sheepshead minnows exposed to a lower Kepone concentration (0.8 μg/liter) over a longer duration (11 days). The same authors also noted that growth, reproduction and survival of sheepshead minnows were affected in 36 days by Kepone concentrations as low as 0.08 μg/liter, which is 0.001 of our sheepshead minnow LC50 (169.5 μg/liter). If we assume that the same ratio exists for spot as Hansen et al. reported for sheepshead minnows, then the no-effect level for spot would be less than 0.0017 μg/liter based on our spot LC50 (6.6 μg/liter).

No color changes were observed in the test crustaceans although they also were lethargic in Kepone concentrations greater than 75 μg/liter.

Kepone was bioconcentrated by all test animals in 96 hours, although bioconcentration factors (concentration of Kepone in tissue divided by measured Kepone in water) varied between species (Table 1). (The bioconcentration factors for fish were similar, 5 × 12 to 15 × 80). The two fishes bioconcentrated Kepone an average of 1.7 to 2.2 times the bioconcentration factor for the grass shrimp and 150 to 190 times that measured in the blue crab. This difference in bioconcentration has been noted in similar bioassays with other organochlorine insecticides. Schimmel et al. (1976) reported that sheepshead minnows, spot, and pinfish (*Lagodon rhomboides*) bioconcentrated 4 to 70 times more heptachlor than grass shrimp or pink shrimp (*Penaeus duorarum*). A similar relationship occurred when some of the same species were exposed to toxaphene (Schimmel et al., in press) and dieldrin (Patrick et al. 1974) in 96-hour bioassays. The reason for an extremely low bioconcentration factor in the blue crab compared to grass shrimp is not known.

Further studies over a longer period of time are required to better understand the more subtle effects of Kepone on estuarine animals. One reason for this assessment is that most deaths occurred after 48 hours in our tests. These studies should include: (1) long-term bioconcentration studies; (2) bioassays which include the hatching and early development of an estuarine animal such as spot; and (3) studies to determine movement of Kepone through an estuarine food web.

Acknowledgments

The authors wish to recognize the significant contributions of Jerrold Forester and Johnnie Knight in the chemical analyses of water and tissue samples and of James M. Patrick, Jr., for help in the bioassays.

Literature Cited

American Public Health Association. 1971. Standard Methods for the Examination of Water and Wastewater. American Public Health Association. 14th ed. Washington, D.C. 1193 p.

Butler, P. A. 1963. Pesticide-Wildlife Studies - A Review of Fish and Wildlife Service Investigations During 1961 and 1962. U.S. Fish and Wildlife Circular. 167 pp. 11–25.

Finney, D. J. 1971. Probit Analysis. Cambridge University Press, Great Britain. 333 p.

Lowe, J. I., P. R. Parrish, J. M. Patrick, Jr., and J. Forester. 1972. Effects of the polychlorinated biphenyl Aroclor® 1254 on the American oyster, *Crassostrea virginica*. *Mar. Biol. (Berlin)* 17:209–214.

Hansen, D. J., L. B. Goodman and A. J. Wilson, Jr. 1977. Kepone: Chronic effects on embryos, fry, juvenile and adults sheepshead minnows, *Cyprinodon variegatus*. *Chesapeake Sci.* 18 (3):337–343.

Parrish, P. R., J. A. Lucas, J. Forester, J. M.

Patrick, Jr., and H. H. Cleve. 1974. Herbicide Effects on several estuarine organisms. Proc. 27th Annu. Conf. Southeastern Assoc. Game Fish Comm. 427–434.

Schimmel, S. C., J. M. Patrick, Jr., and J. Forester. 1976. Heptachlor: Toxicity to and uptake by several estuarine organisms. *J. Toxicol. Environ. Health* 1:933–963.

———, J. M. Patrick, Jr., and J. Forester. Uptake and toxicity of Toxaphene in several estuarine organisms. *Arch. Environ. Contam. Toxicol.* (in press).

Steven C. Schimmel
AND
Alfred J. Wilson, Jr.
U.S. Environmental Protection Agency
Environmental Research Laboratory
Gulf Breeze, Florida 32561